60 Biocide resistance in the multidrug resistant cystic fibrosis pathogens *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex

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Pseudomonas aeruginosa and the *Burkholderia cepacia* complex represent very problematic cystic fibrosis pathogens with innate multidrug resistance. We investigated antimicrobial resistance in these pathogens, specifically focusing on biocide resistance. Biocides such as chlorhexidine and cetylpyridinium chloride are widely used for disinfection of the hospital and home environments. Biocide resistance was determined for panels of representative *P. aeruginosa* and *B. cepacia* complex strains (23 and 81 strains, respectively). Strains were chosen based on their Multilocus Sequence Type and clinical background such as transmissibility. Minimum inhibitory concentrations (MICs) for chlorhexidine and cetylpyridinium chloride were determined using broth dilution assays. *B. cenocepacia* was significantly more resistant to chlorhexidine than other species within the complex (mean MIC = 54.5 μg/ml). *P. aeruginosa* strains had significantly lower chlorhexidine resistance (mean MIC = 18.64 μg/ml) than *B. cenocepacia* strains. No correlation between biocide resistance and antibiotic resistance was observed; E.g, the B. dolosa strain LMG 18943 had low resistance to chlorhexidine (25 μg/ml), but was resistant to all ten antibiotics tested. A microarray experiment was carried out to determine what genes are expressed by *B. cenocepacia* J2315 in response to exposure to chlorhexidine. Significant up-regulation of expression was seen in: (i) multidrug efflux pump genes; (ii) LPS biosynthesis genes; and (iii) protein secretion genes; flagellar biosynthesis genes were down-regulated.

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61* Photodynamic therapy against antibiotic resistant *Pseudomonas aeruginosa* isolates from CF patients

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Background: Photodynamic therapy (PDT) combines a photosensitizer dye with visible light to excite the dye to generate reactive oxygen radicals toxic to cells. The emergence of antibiotic resistance in *Pseudomonas aeruginosa* (PA) CF isolates highlights the need for alternative treatment strategies. This study is aimed to evaluate the in-vitro effect of PDT alone and in the presence of antibiotics on CF PA isolates.
Methods: PA ATCC 27853, PAOmutS and 4 CF PA isolates were tested for susceptibility against ceftazidime (CAZ), meropenem (MEM), amikacin (AK) and ciprofloxacin (CIP) by microdilution method. PDT was applied by using toluidine blue at a light dose of 70 joule/cm2 with a light emitting diode and light mediated killing was determined by viable cell counts. PDT was also applied on antibiotic containing MIC trays and effect on MIC values were tested. MBC values were determined by direct plate count for MIC and MIC+PDT trays.
Results: PDT alone exhibited ⩾2 log killing in 3, 1 log in one and no effect in 2 strains. PDT application in presence of antibiotics lowered the MIC values significantly except for MEM. PDT application in the presence of AK and CIP decreased viable cell counts at least 3 logs for all the strains. PDT+CAZ and PDT+MEM achieved bactericidal activity in half of the strains.
Conclusions: Although PDT alone was not able to achieve bactericidal concentrations for all the tested strains, a significant bactericidal effect was detected when PDT was applied in the presence of antibiotics. Resistance to antibiotics in CF PA isolates may be overcome by the synergistic activity of PDT which should further be improved for in-vivo application.

62 Potential use of photodynamic antimicrobial therapy for treatment of *Pseudomonas aeruginosa* Cystic Fibrosis pulmonary infection

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Introduction and Aims: Photodynamic antimicrobial chemotherapy (PACT), in which a combination of a photosensitiser and visible light cause selective destruction of microbial cells could be a potential alternative method for treatment of chronic Cystic Fibrosis (CF) pulmonary infection caused by bacteria such as *P. aeruginosa*. The aim of this in vitro study was, therefore, to investigate the potential use of PACT in the treatment of *P. aeruginosa* CF pulmonary infection.
Methods: The diffusion of two photosensitisers (toluidine blue O [TBO]) and meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate (TMP) and light across artificial mucus was determined using a modified Franz cell and a light detector, respectively. The susceptibility of clinical *P. aeruginosa* isolates growing both planktonically and in biofilm to PACT was determined using microtitre tray assays.
Results: Red light (635 nm) and both TBO and TMP were delivered successfully across artificial CF mucus with similar concentrations of both photosensitisers achieved in the receiver compartment to those required to achieve high rates of kill (>99%) of *P. aeruginosa* isolates growing both planktonically and in biofilms. TMP required significantly higher concentrations (2.5 mg/ml) than TBO to achieve high rates of kill (>99%) of *P. aeruginosa* isolates growing planktonically. Higher concentrations (5.0 mg/ml) of both photosensitisers were required to achieve high rates of kill (>99%) of *P. aeruginosa* isolates growing in biofilms.
Conclusion: PACT is capable of achieving high levels of kill of clinical *P. aeruginosa* isolates growing both planktonically and in biofilm.

63 Use of tigecycline to treat difficult respiratory pathogens in cystic fibrosis – early experiences

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Tigecycline is a new injectable glycylcycline antibiotic related to minocycline. It has in-vitro activity against non-fermentative Gram negative bacteria (excluding *Pseudomonas aeruginosa*), *Staphylococcus aureus* (including MRSA), *Haemophilus influenzae*, and rapid-growing non-tuberculous mycobacteria. We report our early experiences of using it to treat difficult respiratory pathogens in cystic fibrosis (CF). Since June 2006 we have treated six patients with CF with intravenous tigecycline (four adults, two children aged 12 and 16) using a 100 mg loading dose followed by 50 mg twice daily. The targeted pathogens were *Burkholderia cepacia* complex, *Pandoraea apista, Stenotrophomonas maltophilia, Mycobacterium abscessus,* and *M. chelonae*. Treatment options in all six cases were limited by multi-resistance and/or allergy to conventional therapies. Two patients discontinued tigecycline early (after five and seven days, respectively) because of nausea and vomiting. The other four patients tolerated the drug after they were given prophylactic anti-emetics, with courses ranging from 11 to 33 days. All four showed evidence of clinical response, although co-adminstration of other therapies (mainly beta-lactams and aminoglycosides) may also have contributed to this. Tigecycline is a promising addition to the often-limited treatment options available for these pathogens, but uncertainty over stability have prevented its use outside of the in-patient setting. Properly constructed trials are required to ascertain its true clinical value.